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Activating MAP KAP Kinase 2

The crystal structures of MAP KAP kinase 2 in complex with ADP and staurosporine suggest a major reorganization of the glycine-rich loop upon ligand binding. The main determinant for the catalytic activity of MAP KAP kinase 2 is phosphorylation by p38 MAP kinase.

Anti-inflammatory therapy is a multibillion-dollar global market that has received significant attention from the pharmaceutical industry. The COX-2 inhibitors Celebrex and Vioxx were a major improvement in the treatment of rheumatoid arthritis, and worldwide sales of the two drugs are expected to reach $9.5 billion in 2005 (Renfrey et al., 2003). Not surprisingly, other anti-inflammatory targets are actively being pursued. The p38 mitogen-activated protein (MAP) kinase pathway plays an important role in inflammation (Salituro et al., 1999). Several inhibitors of p38 MAP kinase are in clinical trials for the treatment of inflammation, acute coronary syndrome, rheumatoid arthritis, chronic obstructive pulmonary syndrome, and psoriasis. MAP KAP kinase 2 (MK2) is also a member of the p38 MAP kinase pathway, with p38 MAP kinase as its main cellular activator. The p38/MK2 pathway mediates stress response and is activated by heat shock, UV, lipopolysaccharides, and pro-inflammatory cytokines (Cuenda et al., 1995).

A knockout study in mice provided important proof that MK2 is a potential target for anti-inflammatory therapy (Kotlyarov et al., 1999). More than 50% of knockout mice survived endotoxic shock with LPS/galactosamine in comparison to only 11% of wild-type mice. The *MK2*$^{-/-}$ mice release lower levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-10, and IFN-γ. The regulation of the levels of these cytokines and other proteins such as COX-2 is at the mRNA level but involves different mechanisms. The amount of TNF-α is regulated through translational control via the AU-rich elements of the 3′-UTR of TNF-α mRNA (Neininger et al., 2002). MK2 signaling increases TNF-α mRNA translation. In the case of IL-6, COX-2, and macrophage inflammatory protein 2, MK2 signaling leads to increased mRNA stability (Winzen et al., 1999; Lasa et al., 2000; Neininger et al., 2002).

MK2 is located in the nucleus, but moves rapidly to the cytoplasm after stimulation (Engel et al., 1998; Ben-Levy et al., 1998). It has substrates both in the nucleus (CREB; Tan et al., 1996) and in the cytoplasm (leukocyte-specific protein-1 and heat shock proteins HSP25/27; Huang et al., 1997; Stokoe et al., 1992). A recently discovered substrate of MK2 is heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0) (Rousseau et al., 2002). This protein was captured from the extracts of a murine macrophage-like cell line using the AU-rich element of TNF-α mRNA and is therefore a direct link between MK2 and pro-inflammatory cytokine response. HnRNP A0 also binds the AU-rich elements of MIP-2 and COX-2, and the binding is enhanced when hnRNP A0 is phosphorylated by MK2. MK2 not only transmits p38 signaling to downstream components of the p38 MAP kinase pathway, it also determines the cellular location of p38. MK2 has a nuclear localization signal close to its carboxyl terminus and a nuclear export signal that is part of the autoinhibitory domain (Engel et al., 1998; Ben-Levy et al., 1998). The crystal structure of the autoinhibited MK2 revealed how the protein deals with those two signals (Meng et al., 2002). In nonstressed cells, the autoinhibitory domain binds in the substrate binding groove of the MK2 catalytic domain. In this state, the nuclear export signal is buried between the catalytic and autoinhibitory domains, but the nuclear localization signal is exposed. When the cell undergoes stress, MK2 and p38 migrate to the cytoplasm. This migration, however, occurs only when p38 is catalytically active. The key event here is the phosphorylation of MK2 residue 334, which is located at the N-terminal end of the autoinhibitory domain. Phosphorylation at this residue, together with the phosphorylation of T222 in the activation loop of MK2 by p38, is thought to release the autoinhibitory domain and expose the nuclear export signal. Catalytically incompetent mutant p38 or inhibited p38 will prevent the MK2-p38 complex from moving to the cytoplasm (Ben-Levy et al., 1998). Because the catalytic activity of p38 is required to respond to stress, it is important to note that the activators of p38, MKK3, and MKK6 are localized in the nucleus and cytoplasm. This is in contrast to the MEK1/ERK signaling route, where MEK1 is confined to the cytoplasm and activated ERK moves from the cytoplasm to the nucleus.

MK2 is not the only kinase whose catalytic activity is regulated by a C-terminal autoinhibitory segment. Calcium/calmodulin-dependent kinase-I (cAMK-I), titin kinase domain, and twitchen kinase domain also have a C-terminal tail that folds on the kinase domain and inhibits the catalytic activity of the kinases (Goldberg et al., 1996; Mayans et al., 1998; Kobe et al., 1996). In all those instances, the autoinhibitory segment consists of multiple helices that at least partially block the substrate binding groove. In the case of cAMK-I, the C-terminal end of the autoinhibitory domain also binds the glycine-rich loop of the β strand domain, blocking the entrance of the ATP binding site. For the titin and twitchen kinase domains, part of the autoinhibitory segment occupies the ATP binding site. A short helical segment sits under the glycine-rich loop, preventing ATP from binding. For MK2, the autoinhibitory domain mimics substrate binding and completely occupies the substrate binding groove. In order for these kinases to become catalytically active, the autoinhibitory domain must be dissociated from the kinase domain. This is a two-step activation process for all four kinases. For CaMK-1, titin kinase, and twitchen kinase, the first step is the binding of Ca/calmodulin to the autoinhibitory domain. The second step is the phosphorylation of an activation loop residue on the kinase domain. For MK2, the first step is the phosphorylation of T334 at the far N-terminal end of the autoinhibition segment followed by phosphorylation of T222 on the activation loop.

In this issue of *Structure*, Underwood et al. present the crystal structures of catalytically active MK2 in complex with ADP and staurosporine, together with a detailed kinetic analysis that explores the mechanism of regulation of MK2. The ligand-bound structures are significantly different from the autoinhibited structure. The ATP binding site of autoinhibited MK2 is not suitable for ATP binding, but the work presented here makes clear how MK2 facilitates this. The binding of the ligand induces a reorganization of the glycine-rich loop and changes from a helix-loop-β strand to an antiparallel β strand as seen in other kinases. Ligand binding induces not only the unwinding of the helix but also rotates the backbone approximately 60° to cover the ATP binding site. The autoinhibitory domain binds as a pseudosubstrate to the kinase domain with no significant effect on ATP binding. Partial truncation of the autoinhibitory domain allows LSP-1 peptide to bind weakly. However, Underwood et al. (2003) have shown that phosphorylation by p38 is the major determinant for tight binding and phosphorylation of substrates. Now that the crystal structures of the activated and autoinhibited form of MK2 are known, the stage is set for a thorough structure-based drug design effort. It would be interesting to see whether MK2 inhibition, as its biology suggests, becomes a new and better way to treat inflammatory diseases.

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A Growing Family of Natural Killers

The structure of the natural cytotoxicity receptor NKp44, described in this issue of *Structure*, adds to our rapidly expanding knowledge of the structure of natural killer cell receptors, which play a key role in the elimination of virally infected and tumor cells during innate immune responses.

Natural killer (NK) cells are a fundamental component of the innate immune system that participate in the early detection and destruction of virally infected and tumor cells (Biron et al., 1999). The cytolytic activity of NK cells is regulated through a delicate balance between inhibitory and stimulatory signals delivered by NK receptors that recognize diverse ligands on target cells. The dominant signal received by an NK cell through its interaction with normal levels of major histocompatibility complex class I (MHC-I) molecules on target cells is inhibitory. When the level of MHC-I is reduced through infectious or tumorigenic processes, this inhibitory signal is attenuated, and the NK cell is triggered through its activating receptors, which include NKp44. In this way, diseased cells with abnormal MHC-I expression become the targets of NK lytic activity resulting from the loss of inhibition of NK cell activation.

NK receptors belong to two structurally distinct families comprising C-type lectin-like domain-containing molecules with type II membrane topology (e.g., Ly49s, NKG2D, CD94/NKG2, NKR-P1, CD69), and proteins displaying an immunoglobulin (Ig) fold with type I membrane topology (e.g., KIRs, LIRs, NKp46, 2B4) (Natarajan et al., 2002). Both the C-type lectin-like and Ig-like families include inhibitory and activating receptors. Some of